Use of Spectral Vegetation Indices Derived from Airborne Hyperspectral Imagery for Detection of European Corn Borer Infestation in Iowa Corn Plots

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ABSTRACT Eleven spectral vegetation indices that emphasize foliar plant pigments were calculated using airborne hyperspectral imagery and evaluated in 2004 and 2005 for their ability to detect experimental plots of corn manually inoculated with *Ostrinia nubilalis* (Hübner) neonate larvae. Manual inoculations were timed to simulate infestation of corn, *Zea mays* L., by first and second flights of adult *O. nubilalis*. The ability of spectral vegetation indices to detect *O. nubilalis*-inoculated plots improved as the growing season progressed, with multiple spectral vegetation indices able to identify infested plots in late August and early September. Our findings also indicate that for detecting *O. nubilalis*-related plant stress in corn, spectral vegetation indices targeting carotenoid and anthocyanin pigments are not as effective as those targeting chlorophyll. Analysis of image data suggests that feeding and stem boring by *O. nubilalis* larvae may increase the rate of plant senescence causing detectable differences in plant biomass and vigor when compared with control plots. Further, we identified an approximate time frame of 5–6 wk postinoculation, when spectral differences of manually inoculated "second" generation *O. nubilalis* plots seem to peak.

KEY WORDS Bt corn, remote sensing, *Ostrinia nubilalis*, European corn borer, resistance

In 2006, field corn, *Zea mays* L., grown for grain was harvested from >28 million ha in the United States and had a production value of >$33 billion (NASS 2007a,b). One of the most serious pests to corn production in North America is the European corn borer, *Ostrinia nubilalis* (Hübner). *O. nubilalis* is present throughout the major corn-producing areas in the United States, and it is capable of significantly reducing corn yield if not controlled (Ostlie et al. 1997). *O. nubilalis* is capable of multiple generations per year and in states like Iowa, the *O. nubilalis* ecotype is bivoltine, with two distinct moth flights each of 4–6-wk duration (Showers et al. 1975).

Transgenic corn expressing the insecticidal plant incorporated protectant (PIP) *Bacillus thuringiensis* (Bt) toxin was commercially released for *O. nubilalis* control in 1996 and accounted for 40% of all U.S. corn planted in 2006 (NASS 2007b). Transgenic corn hybrids currently available to growers contain multiple traits (stacks), including Bt for corn borer [*O. nubilalis* and *Diatraea grandiosella* (Dyar) control], Bt for corn rootworm (*Diabrotica virgifera virgifera* LeConte and *Diabrotica barberi* Smith & Lawrence) control and herbicide tolerance. The U.S. Environmental Protection Agency (U.S. EPA) registers all PIP-expressing plants under the Federal Insecticide Fungicide and Rodenticide Act and has determined that crops containing insecticidal PIP traits are beneficial to the public because of their potential to increase yields with fewer applications of insecticides (U.S. EPA 2001, Hunt et al. 2007). However, widespread use of these crops increases risk of corn insect pests developing resistance to Bt toxins. Loss of Bt trait effectiveness due to insect resistance would increase grower reliance on broad-spectrum chemical insecticides that would, in turn, reduce environmental quality, and increase worker exposure to hazardous chemicals.

To minimize potential for development of insect resistance to Bt corn, U.S. EPA mandates a high-dose/refuge insect resistance management (IRM) strategy for *O. nubilalis*. This management tactic is based on the assumptions that plants are expressing Bt toxin at levels sufficient to ensure that resistance is functionally recessive and any potentially resistant *O. nubilalis* are most likely to mate with susceptible moths emerg-

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ing from non-Bt corn refuge (Tabashnik and Croft 1982; Alstad and Andow 1995, 1996; Roush 1997; Gould 1998). Depending on regional crop composition, U.S. EPA requires that growers plant 20 to 50% of their acreage to non-Bt corn hybrids to ensure adequate production of susceptible moths. Additionally, U.S. EPA requirements for PIP crop registration include stewardship responsibilities, such as crop monitoring for insect pest resistance. However, a national monitoring program designed to detect early development of *O. nubilalis* resistance to Bt corn by using traditional sampling methods requires physically sampling cornfields on a geographic scale that would be logistically impractical and expensive to implement.

Remote sensing provides a means of reliably locating corn plantings in the landscape and detecting crop plant stress (Bauer et al. 1979, Bauer 1985, Curran 1985, Lichtenthaler et al. 1998, Zhao et al. 2005, Tilling et al. 2007). It potentially could improve upon current ground-based techniques for monitoring *O. nubilalis* in corn by providing spatially explicit information on the entire field that can be integrated with existing *O. nubilalis* sampling methodology. Additionally, the flexibility offered by numerous aerial and satellite platforms allows remote-sensed imagery at different spectral and spatial resolutions to be acquired nearly simultaneously over time (Cihlar et al. 1991). Consequently, the areal scalability and spatial context of remote-sensed imagery would address many logistical issues facing a national monitoring program for *O. nubilalis* in field corn. Imagery acquired by satellites at regional scales would allow discrimination of corn from other crops in the landscape, whereas higher resolution airborne hyperspectral images would allow evaluation of individual corn fields for hybrid type and insect-related plant stress.

Hyperspectral sensors, sometimes referred to as imaging spectrometers, provide a high degree of spectral resolution compared with multispectral imagery by taking nearly continuous measurements over the visible, near infrared (IR) / middle IR regions of the spectrum at narrow intervals. The increased sensitivity provided by the spectral resolution of hyperspectral imagery allows unique spectral features associated with crop condition to be identified (Thenkabail et al. 2000, Govender et al. 2007). However, the improved spectral resolution of hyperspectral imagery significantly increases image dimensionality, data redundancy, and file size. Spectral vegetation indices (SVI), such as the normalized difference vegetation index (NDVI) (Rouse et al. 1974, Tucker 1979), provide one method of reducing multidimensional data acquired from multiband or hyperspectral imagery to a single value that can be used to qualitatively and quantitatively assess various crop vegetation parameters while maintaining unique spectral information (Perry and Lautenschlager 1984, Apan et al. 2004).

Conceptually, use of remote sensing to indirectly evaluate crop condition is not new and benefits of early detection of crop stress are obvious but examples of its practical employment are limited. However, the increasing number of ground, aerial and satellite based sensors available for use, improved spatial and spectral resolution of current sensors and integration of accurate spatial information through global positioning system (GPS) is making remote sensing more applicable to agricultural science. Use of remote sensing to assess crop parameters, such as nitrogen, iron, and phosphorous status (Daughtry et al. 2000, Osborne et al. 2002, Zhao et al. 2003, Schlemmer et al. 2005), and biophysical information, such as leaf area index (LAI) and yield (Thenkabail et al. 2000, Calera et al. 2004, Haboudane et al. 2004, Vina et al. 2004), are becoming more common. Remote sensing also has been used in agricultural insect pest management. Recent examples include use of remotely sensed imagery to examine crop–insect interactions at local and regional scales (Brewster et al. 1999, Grilli 2006), investigate feeding damage to winter wheat, by greenbug (Mirik et al. 2006), detect cotton aphid and spider mite damage to cotton (Fitzgerald et al. 2004, Reisig and Godfrey 2006), examine chlorophyll loss from soybean aphid feeding on soybean (Diaz-Montano et al. 2007), characterize reflectance spectra of wheat infested with Russian wheat aphid and greenbug (Riedell and Blackmer 1999) and detect phylloxera-infested grape vines (Blanchfield et al. 2006). Much of this research was conducted using spectroradiometers with small fields of view (FOV) of less than a meter or used satellite imagery with coarse spatial resolutions, both of which can limit their usefulness for insect pest management in production agriculture. However, these studies demonstrate the feasibility of using spectral detection methods for identification of insect related plant stress.

Use of spectral information as an indicator of plant stress requires an alteration of the plants reflectance characteristics in visible light between 380- and 700-nm wavelengths and in the infrared at wavelengths between 700 and 2500 nm (Carter 1993). Physical invasion of corn stalks by *O. nubilalis* larvae disrupts nutrient and water translocation within the corn plant, increases incidence of stem rot diseases, such as anthracnose (Bergstrom and Nicholson 1999), and can induce early senescence. Disruption of water and nutrient translocation influences plant pigment dynamics and is well correlated with a plant's physiological condition (Blackburn 2007). Leaf water content influences light absorption by determining how light interacts with wavelength independent factors, such as internal leaf structures that are defined by the type and number of cells present, and their distribution, shape, and ultrastructure (Woolley 1971, Gausman 1977, Carter 1991). Changes in reflectance from disruption of water translocation are caused by increased numbers of interfaces between wet cells and intercellular air spaces that develop as water is lost from plant tissues (Gausman and Cardenas 1969, Knipling 1970, Woolley 1971, Carter 1991). Typically, leaf water content in most plants influences light reflectance at wavelengths >1,000 nm (Carter 1991, Broge and Leblanc 2000), but as Woolley (1971) cautioned when explaining the influence of leaf water content on light reflectance for soybean, *Glycine max* (L.) Merr., and

corn leaves, the amount of change observed in the intercellular spaces may depend on differences in mesophyll (i.e., monocots and dicots) and photosynthetic capacity. *O. nubilalis* larvae feeding within the plant stem should affect adaxial and abaxial surface reflectance between 400 nm and 2,500 nm as a result of decreased water content in plant tissues (Woolley 1971, Carter 1991) and be detectable by a hyperspectral sensor. Additionally, yellowing and other visible color changes associated with stressed or senescing leaf tissue that results from chlorophyll degradation unmasking secondary pigments such as carotenoids and anthocyanins (Matile and Hortensteiner 1999) also may be detectable.

The objective of this study was to determine whether spectral vegetation indices using wavelengths sensitive to the plant pigments chlorophyll, anthocyanin, and carotenoids, are able to distinguish corn plots manually inoculated with *O. nubilalis* from noninoculated control plots of corn. We discuss use of pigment-based indices and their potential role in monitoring for *O. nubilalis* resistance to Bt corn.

Materials and Methods

Experimental Plots. Experimental corn plots were planted southwest of Ames, IA, on the Bennett farm in 2004 and Been farm in 2005 by using standard grower practices for corn production. Nitrogen applied at 157 kg/ha in liquid 32% form was tank mixed with the herbicide Acetochlor (2-chloro-*N*(ethoxymethyl)-6′-ethyl-*o*-acetotoluidide) and applied to all plots in 2004 and 2005 before plant emergence. A postemergence herbicide, Diglycolamine salt (3,6-dichloro-*o*-anisic acid) was applied later in the season. Row spacing for all plots in 2004 and 2005 was 76.2- at 19.1-cm plant spacing. Individual plots were 4.6 m (four rows) by 18.3 m in 2004 but were increased to 9.1 m (12 rows) by 18.3 m in 2005 to increase the number of picture elements (pixels) available for image analysis. Inoculation treatments were randomly assigned to plots of DeKalb DKC57-01 field corn by using a randomized block design replicated five times in 2004 and 2005.

Inoculation of Corn Plants with *O. nubilalis*. Plants were inoculated with *O. nubilalis* neonates (Guthrie and Barry 1988) during V8-V10 growth stages on 24 and 25 June 2004 and 22 and 24 June 2005 to simulate first generation *O. nubilalis* and during R1, or R2 growth stages on 27 and 28 July 2004 and 22 July 2005 to simulate second generation *O. nubilalis* (Ritchie et al. 1997). In 2004, three treatments were established using 50 *O. nubilalis* neonates per plant for first generation, 50 neonates per plant for second generation, and plots with no inoculation to serve as a control. In 2005, an additional *O. nubilalis* density of 25 neonates every second plant, categorized as low, was added to second generation *O. nubilalis* treatments. Four and eight middle rows of treated plots were inoculated in 2004 and 2005, respectively.

Damage Assessment. Foliar feeding by first generation *O. nubilalis* larvae was evaluated using the Guthrie rating scale on 8 July 2004 when plants were at V12-V13 growth stage and 7 and 8 July 2005 at the V9-V11 growth stage. The mean Guthrie rating for 10 total plants was calculated for each plot by arbitrarily selecting five consecutive plants from each of the two center rows and visually assessing leaf feeding on a 1–9 scale where one is no damage and nine is extensive damage (Guthrie and Barry 1988). Stalk tunneling damage by *O. nubilalis* was assessed at the end of the growing season in 2004 and 2005 by arbitrarily selecting five consecutive plants from each of the two center rows for dissection. Plants were dissected by vertically splitting each stem along the length of the stalk with a hawk billed grafting knife (pruner #1B, Camillus Cutlery. Camillus, NY). Plants were individually evaluated for damage from *O. nubilalis* tunneling by enumerating number of entrance holes resulting in a tunnel >1 cm. Total number of cavities per plant were recorded and converted to mean number of cavities per 10 plants for a given treatment.

Hyperspectral Remote Sensing. Hyperspectral images of the experimental plots were acquired at 0.5-m resolution by using a real-time data acquisition camera system-hyperspectral (RDACS-H4) airborne camera system modified by ITD (Institute of Technology and Development, Savoy, IL) mounted on a Zeiss T-A1 gyro-stabilized suspension system (Intergraph, Huntsville, AL) in a Cessna 210 single engine fixed wing aircraft. Plots were imaged six times in 2004 on 12 July, 25 July, 9 August, 20 August, and 3 September and 17 September and five times in 2005 on 1 August, 17 August, 30 August, 10 September, and 26 September. Spectral resolution of images consisted of 120 spectral bands between 473 and 827 nm at 0.5-m^2 spatial resolution for 2004 and 240 bands from 400 to 1000 nm at 0.5 m^2 for 2005.

Before analysis hyperspectral imagery was preprocessed to remove signal noise, calibrated to relative reflectance, and then straightened and spatially registered to a real-world coordinate system. Preprocessing was accomplished using ENVI 4.0 (ITT Visual Information Solutions, Boulder, CO) to remove electronic noise by means of a minimum noise fraction (MNF) rotation. The MNF procedure uses both raw hyperspectral and dark current image data as inputs to an algorithm similar to principal components analysis to perform a two cascaded transformation on image data to isolate signal noise.

Calibration of hyperspectral imagery to relative reflectance was achieved using the following methods. Seven 4.57-m^2 gray scaled (black to white) calibration tarps that varied in percent of reflectance from 2 to 83 ± 1% were placed adjacent to the experimental area. A GER 1500 spectroradiometer with a spectral range of 350–1,050 nm at 1.5-nm bandwidth (Geophysical Environmental Research Corp., Millbrook, NY) was used to acquire four scans over quadrants of each tarp as close to the time of image acquisition as was possible. After acquiring plot images, the empirical line method (Smith and Milton 1999) in ENVI 4.0 was used to develop an equation for the relationship between digital numbers recorded by the sensor for

Table 1. Spectral vegetation indices used to differentiate 2004 and 2005 Iowa corn plots manually inoculated with *O. nubilalis* neonate larvae

Index	Abbreviation	Equation	Source
Normalized difference vegetation index	NDVI	$\frac{R_{800} - R_{670}}{R_{800} + R_{670}}$	Rouse et al. (1974)
Red edge position	Red edge	$\frac{R_{670} + R_{780}}{2}$	Guyot and Baret (1988)
Pigment specific simple ratio for chlorophyll a	$PSSR_a$	$\frac{R_{800}}{R_{680}}$	Blackburn (1998)
Pigment specific simple ratio for chlorophyll b	$PSSR_b$	$\frac{R_{800}}{R_{635}}$	Blackburn (1998)
Modified chlorophyll absorption ratio index	MCARI	$((R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550})) * \left(\frac{R_{700}}{R_{670}}\right)$	Daughtry et al. (2000)
MCARI/OSAVI ratio	MOSR	$\frac{((R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550})) * \left(\frac{R_{700}}{R_{670}}\right)}{\frac{((R_{800} - R_{670}) - 1)}{((\sqrt{R_{800} + R_{670}}) + 1)}}$	Haboudane et al. (2002)
Anthocyanin reflectance index (ARI)	ARI	$(R_{561}^{-1} - R_{706}^{-1}) * R_{780}$	Gitelson and Merzlyak (2004)
Carotenoid reflectance index, green wavelength	CRI_{green}	$(R_{510}^{-1} - R_{561}^{-1}) * R_{780}$	Gitelson and Merzlyak (2004)
Carotenoid reflectance index, red wavelength	CRI_{red}	$(R_{510}^{-1} - R_{706}^{-1}) * R_{780}$	Gitelson and Merzlyak (2004)
Green model	GM	$\left(\frac{R_{747}}{R_{549}}\right) - 1$	Gitelson et al. (2005)
Red model	RM	$\left(\frac{R_{747}}{R_{700}}\right) - 1$	Gitelson et al. (2005)

the calibration tarp and the "true" reflectance values from spectroradiometer scans.

Before spatial registration of the imagery, a spatial reference grid for the experimental plots was created by placing 30 white painted 0.25-m^2 wooden panels midway between plots and at plot corners along the exterior. Panels were georeferenced to centimeter accuracy using a Trimble Pathfinder ProXRS GPS unit (Trimble Navigation Limited, Sunnyvale, CA) and digitally stored for later use.

Spatial registration of acquired images to real world coordinates was accomplished using two methods. Preliminary image rectification was conducted using HYPER, a proprietary program developed by Institute for Technology Development (ITD) (Stennis Space Center, MS) that removes roll distortion in the imagery caused by aircraft roll motion. Imagery was georectified using ERDAS Imagine 8.7 (Leica Geosystems Geospatial Imaging, LLC, Norcross, GA). Additionally, hyperspectral imagery needed to be subset so that pixels with mixed reflectance from between plots and bordering areas would be excluded. Polygon shapefiles were created for each plot and used to define individual plot areas for export. Each hyperspectral image was converted from image space to ASCII format by using ERDAS Imagine's convert pixel to ASCII utility.

Eleven SVI that target plant pigments were selected for evaluation in this study (Table 1) and were considered a representative subset of many SVI currently available. Calculations for individual SVI (Table 1) were performed in SAS by using the 2004 and 2005 exported hyperspectral imagery data of the Iowa experimental plots. In 2005, some wavelengths used in SVI calculations differed from those used in 2004 by 1 to 2 nm due to the increased spectral resolution of the 2005 imagery, but this difference was considered negligible.

Analyses. All data sets were checked for normality and homogeneity of variance using residual and normality plots before conducting analyses of variance (ANOVA). In cases where assumptions were not met, a $\log(y + c)$, square root$(y + c)$ or y^2 transformation was sufficient to correct the issue, where c represents a constant added to change values of y from negative to positive.

O. nubilalis damage levels in the plots were checked to determine whether differences between inoculation treatments were achieved. Mean Guthrie ratings and mean number of *O. nubilalis* cavities per plant were compared using a single factor ANOVA, Proc Mixed of SAS 9.1.3 (SAS Institute, Cary, NC), at the 0.05 level of significance. A multiple comparison between 2005 second generation *O. nubilalis* high- and low-inoculated treatments and noninoculated controls for mean number of *O. nubilalis* cavities per plant was performed using the Tukey–Kramer method for multiple comparisons ($\alpha = 0.05$).

Comparison of mean spectral vegetation indices per treatment for 2004 and 2005 first and second generation infestations were analyzed with repeated measures ANOVA (Proc Mixed SAS 9.1.3) by using calendar day of image acquisition to represent time beginning at the first imaging date after inoculation. The default variance-component covariate structure used by Proc Mixed (SAS 9.1.3) for analysis of time-series data were not appropriate for imaging events

Table 2. Mean number of *O. nubilalis* cavities per 10 plants from 2004 and 2005 Iowa corn plots manually inoculated with *O. nubilalis* neonate larvae

Yr	*O. nubilalis* generation	Treatment	Inoculation rate	Mean Guthrie rating ± SE	Mean no. cavities ± SE[a]
2004	First	Control	0	1.66 ± 0.16a	0.02 ± 0.004a
2004	First	High	50 neonates/plant	3.48 ± 0.17b	0.08 ± 0.02b
2004	Second	Control	0		0.02 ± 0.004a
2004	Second	High	50 neonates/plant		0.76 ± 0.05b
2005	First	Control	0	1.80 ± 0.53a	0.02 ± 0.01a
2005	First	High	50 neonates/plant	4.11 ± 0.48b	0.09 ± 0.03a
2005	Second	Control	0		0.02 ± 0.01a
2005	Second	Low	25 neonates every fourth plant		0.1 ± 0.01a
2005	Second	High	50 neonates/plant		0.4 ± 0.03b

[a] Mean number of cavities per 10 plants with the same letter for each year and generation are not statistically different at 0.05 level of significance.

not equally spaced over time. However, temporal measurements, such as imaging events, can be treated as a one-dimensional spatial process. Consequently, the spherical (power) spatial covariate structure was used to model covariance between imaging events acquired at different times (Littell et al. 1996). When treatment-time interactions were significant, differences between mean SVI for *O. nubilalis* inoculated treatments and noninoculated controls were examined by date ($\alpha = 0.05$) by using least square means (LS-means) in Proc Mixed (SAS 9.1.3). For multiple comparisons, differences between mean SVI for different inoculated treatments and noninoculated controls were examined using the adjusted LS-means provided by the Tukey–Kramer method for multiple comparisons ($\alpha = 0.05$) in Proc Mixed (SAS 9.1.3).

Results

Mean Guthrie ratings were found to be significantly different between noninoculated controls and first generation *O. nubilalis* inoculated plots in 2004 ($F = 60.23$; df = 1, 4; $P = 0.001$) and 2005 ($F = 10.47$; df = 1, 4; $P = 0.03$) (Table 2). Significant differences also were found between mean number of *O. nubilalis* cavities per plant (Table 2) for noninoculated controls and first ($F = 16.71$; df = 1, 4; $P = 0.02$) and second ($F = 222.9$; df = 1, 4; $P = 0.0001$) generation *O. nubilalis* inoculated plots in 2004; however, cavity numbers for 2005 (Table 2) first generation *O. nubilalis* inoculated treatments were not significantly different from noninoculated controls ($F = 5.43$; df = 1, 4; $P = 0.08$). Main treatment effect on mean number of cavities per plant for 2005 second generation *O. nubilalis* inoculation was significant ($F = 63.95$; df = 2, 8; $P < 0.0001$). However, only the high second generation *O. nubilalis* inoculation treatment was significantly different from low inoculation ($t = 8.41$; df = 2, 8; $P < 0.0001$) and noninoculated controls ($t = 10.76$; df = 2, 8; $P < 0.0001$) with no significant differences between low inoculation treatments and noninoculated controls ($t = 2.35$; df = 2, 8; $P = 0.11$) (Table 2). Overall, manual inoculation of corn plants with neonate *O. nubilalis* larvae to create plots with different levels of *O. nubilalis* feeding damage was successful.

Eleven SVI were individually evaluated during the growing season for their ability to detect plots of corn manually inoculated with *O. nubilalis* in 2004 and 2005. For 2004 first generation *O. nubilalis* inoculated treatments, no significant treatment main effects or time by treatment interactions were detected (Tables 3 and 4)

Table 3. Repeated measures analysis of variance of spectral vegetation indices derived from hyperspectral imagery of *O. nubilalis* first generation treated corn plots and noninoculated controls from 2004 and 2005 Iowa

Index	2004 first generation *O. nubilalis*						2005 first generation *O. nubilalis*					
	Treatment (df = 1, 4)		Time (df = 5, 48)		Treatment × time (df = 5, 48)		Treatment (df = 1, 4)		Time (df = 4, 32)		Treatment × time (df = 4, 32)	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
ARI	0.06	0.820	591.42	<0.0001	1.39	0.2443	0.06	0.8253	830.35	<0.0001	2.88	0.0381
CRI_{green}	0.00	0.9708	330.08	<0.0001	0.75	0.5893	7.99	0.0475	2550.95	<0.0001	0.34	0.8466
CRI_{red}	0.08	0.7895	316.95	<0.0001	1.42	0.2346	3.53	0.1334	2233.18	<0.0001	0.51	0.7314
GM	0.05	0.8355	396.48	<0.0001	1.24	0.3056	3.64	0.1289	3138.93	<0.0001	2.35	0.0749
MCARI	0.00	1.0000	338.41	<0.0001	0.39	0.8560	0.29	0.6173	1815.26	<0.0001	1.28	0.2972
MOSR	0.01	0.9307	216.35	<0.0001	0.28	0.9241	0.25	0.6435	1247.10	<0.0001	1.02	0.4111
NDVI	0.03	0.8639	668.96	<0.0001	1.10	0.3752	4.94	0.0904	13738.0	<0.0001	5.58	0.0016
$PSSR_a$	0.04	0.8564	488.93	<0.0001	1.28	0.2895	4.63	0.0978	11143.6	<0.0001	3.03	0.0317
$PSSR_b$	0.02	0.9023	521.79	<0.0001	0.90	0.4875	4.38	0.1045	7548.76	<0.0001	4.02	0.0094
Red edge	0.28	0.6254	59.12	<0.0001	1.13	0.3573	10.03	0.0339	162.42	<0.0001	0.70	0.5998
RM	0.04	0.8590	201.77	<0.0001	1.12	0.3642	4.90	0.0912	5076.92	<0.0001	2.81	0.0416

Table 4. Mean spectral vegetation indices for *O. nubilalis* inoculation treatments and noninoculated control by time of image acquisition for first generation *O. nubilalis* inoculated treatments from 2004 Iowa

Date	Days after treatment	*O. nubilalis* generation	Inoculation treatment	ARI	CRI_{green}	CRI_{red}	GM	MCARI	MOSR	NDVI	$PSSR_a$	$PSSR_b$	Red edge	RM
12 July	17	First	Control	1.62a	−0.90a	1.70a	2.21a	6.58a	6.81a	0.73a	1.87a	2.53a	2.99a	1.04a
			High	1.67a	−0.95a	1.73a	2.26a	6.69a	6.90a	0.74a	1.92a	2.58a	3.03a	1.09a
25 July	30	First	Control	1.25a	−0.43a	1.52a	1.90a	5.78a	6.21a	0.68a	1.66a	2.07a	3.17a	0.97a
			High	1.23a	−0.42a	1.51a	1.88a	5.70a	6.16a	0.67a	1.64a	2.04a	3.15a	0.95a
9 Aug.	45	First	Control	1.24a	−0.52a	1.49a	1.88a	6.04a	6.48a	0.67a	1.61a	2.13a	3.10a	0.91a
			High	1.23a	−0.50a	1.48a	1.86a	6.02a	6.49a	0.66a	1.58a	2.10a	3.09a	0.89a
20 Aug.	56	First	Control	1.30a	−0.69a	1.50a	1.90a	6.82a	7.21a	0.68a	1.65a	2.13a	3.08a	0.81a
			High	1.26a	−0.65a	1.49a	1.86a	6.81a	7.25a	0.66a	1.60a	2.10a	3.08a	0.77a
3 Sept.	70	First	Control	0.79a	−0.15a	1.34a	1.52a	4.92a	5.87a	0.51a	1.14a	1.60a	3.05a	0.54a
			High	0.75a	−0.15a	1.32a	1.49a	4.85a	5.83a	0.50a	1.11a	1.57a	3.02a	0.52a
17 Sept.	84	First	Control	0.32a	0.07a	1.20a	1.18a	2.57a	4.19a	0.27a	0.56a	0.79a	2.92a	0.21a
			High	0.33a	0.06a	1.20a	1.19a	2.63a	4.22a	0.28a	0.57a	0.81a	2.90a	0.22a
		SEM		0.04	0.05	0.02	0.03	0.15	0.13	0.02	0.04	0.04	0.02	0.04

Mean SVI with the same letter within each date are not statistically different at 0.05 level of significance.

for any SVI. In 2005, the SVI CRI_{green}, and Red Edge position index both had significant treatment main effects, but neither index had significant treatment by time interactions for first generation inoculation treatments (Table 3). Although most SVI for first generation treatments during 2005 did not have significant main effects several SVI, namely, ARI, NDVI, $PSSR_a$, $PSSR_b$, and Red model had significant time by treatment interactions (Table 3). These indices were able to discriminate first generation inoculated plots from noninoculated controls with three of the SVI, $PSSR_a$, $PSSR_b$ and Red model, able to distinguish between first generation *O. nubilalis* inoculated plots on two consecutive occasions (Table 5).

Plots inoculated with second generation *O. nubilalis* were detected by more SVI than those inoculated with first generation *O. nubilalis* neonates. In 2004, 10 of 11 SVI for second generation *O. nubilalis* inoculated plots had significant main treatment effects, and all had significant treatment by time interactions (Table 6). In contrast, six of 11 SVI had significant main effects in 2005 (Table 6), with five of the six having significant treatment by time interactions.

Further examination of the 2004 LS-means for SVI with significant main treatment effects and treatment by time interactions for second generation *O. nubilalis* inoculated plots (Table 7) shows that mean values of each SVI for control and treatment plots were similar in August, 12 and 23 d after inoculation treatments (DAT). By 3 September (37 DAT), mean SVI for control plots were consistently higher than inoculated plots. Similar trends were observed for 2005 LS-means for six SVI (CRI_{green}, GM, NDVI, $PSSR_a$, $PSSR_b$, and RM) with significant main treatment effects and significant treatment by time interactions (Table 8). Mean SVI for control plots and inoculated plots were not separable until 26 DAT when a significant difference was detected for RM between high infestation *O. nubilalis* treatments and low infestation and noninoculated controls. At 39 and 50 DAT, the six significant SVI showed similar tendencies, with higher mean index values associated with control plots, likely caused by greater plant vigor and biomass compared with inoculated plots. Significant differences between high *O. nubilalis* infestation treatments and low infestation and noninoculated controls were observed at 39 and

Table 5. Mean spectral vegetation indices for *O. nubilalis* inoculation treatments and noninoculated control by time of image acquisition for first generation *O. nubilalis* inoculated treatments from 2005 Iowa

Date	Days after treatment	*O. nubilalis* generation	Treatment	ARI	CRI_{green}	CRI_{red}	GM	MCARI	MOSR	NDVI	$PSSR_a$	$PSSR_b$	Red edge	RM
1 Aug.	38	First	Control	1.69a	1.27a	1.68a	1.70a	1.84a	2.90a	0.73a	2.66a	2.42a	3.11a	2.34a
			High	1.69a	1.25a	1.67a	1.67a	1.85a	2.98a	0.73a	2.63a	2.40a	3.08a	2.30a
17 Aug.	54	First	Control	1.64a	1.55a	1.96a	1.60a[a]	1.95a	3.62a	0.74a	2.60a	2.41a	2.95a	2.26a
			High	1.66b	1.52a	1.95a	1.56a[a]	1.96a	3.80a	0.73a	2.54b	2.36b	2.91a	2.19b
30 Aug.	67	First	Control	1.93a	0.51a[b]	1.35a[c]	1.39a	1.96a	4.38a	0.55a	1.85a	1.89a	2.86a	1.63a
			High	1.92a	0.47a[b]	1.31a[c]	1.34b	1.95a	4.39a	0.52b	1.76b	1.81b	2.80b	1.56b
10 Sept.	78	First	Control	1.95a	1.30a	1.98a	1.27a	2.78a	13.23a	0.70a	2.35a	2.07a	3.06a	1.58a
			High	1.96a	1.27a	1.96a	1.25a	2.77a	13.14a	0.69a	2.31a	2.04a	3.04a	1.54a
26 Sept.	94	First	Control	1.69a	0.46a	0.94a	0.65a	1.14a	−0.80a[d]	0.10a	0.63a	0.74a	2.97a	0.64a
			High	1.68a	0.45a	0.91a	0.63a	1.05b	−1.60a[d]	0.09a	0.61a	0.72a	2.93b	0.64a
		Standard error of mean		0.01	0.01	0.02	0.01	0.03	0.28	0.005	0.02	0.02	0.01	0.02

Mean SVI with the same letter within each date are not statistically different at 0.05 level of significance.
[a] Pr > t = 0.061.
[b] Pr > t = 0.055.
[c] Pr > t = 0.052.
[d] Pr > t = 0.052.

Table 6. Repeated measures analysis of variance of spectral vegetation indices derived from hyperspectral imagery of *O. nubilalis* second generation treated corn plots and noninoculated controls from 2004 and 2005 Iowa

Index	2004 second generation *O. nubilalis*						2005 second generation *O. nubilalis*					
	Treatment (df = 1, 4)		Time (df = 3, 32)		Treatment × time (df = 3, 32)		Treatment (df = 2, 4)		Time (df = 4, 48)		Treatment × time (df = 8, 48)	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
ARI	34.61	0.0042	2185.55	<0.0001	105.62	<0.0001	0.69	0.5541	1191.59	<0.0001	2.80	0.0124
CRI_{green}	6.62	0.0618	1550.48	<0.0001	31.97	<0.0001	9.53	0.0301	4079.31	<0.0001	1.43	0.2100
CRI_{red}	58.55	0.0016	1575.21	<0.0001	114.11	<0.0001	5.26	0.0758	2556.71	<0.0001	1.09	0.3844
GM	29.25	0.0057	2240.58	<0.0001	115.12	<0.0001	17.15	0.0109	5881.57	<0.0001	11.38	<0.0001
MCARI	47.71	0.0023	1192.63	<0.0001	91.16	<0.0001	0.82	0.4946	6923.21	<0.0001	5.47	<0.0001
MOSR	68.02	0.0012	743.32	<0.0001	80.12	<0.0001	1.39	0.3482	2201.31	<0.0001	9.88	<0.0001
NDVI	18.06	0.0132	1529.20	<0.0001	86.99	<0.0001	27.13	0.0047	18156.8	<0.0001	27.50	<0.0001
$PSSR_a$	16.35	0.0156	1896.52	<0.0001	102.45	<0.0001	23.00	0.0064	11767.10	<0.0001	14.86	<0.0001
$PSSR_b$	26.79	0.0066	1722.84	<0.0001	114.74	<0.0001	24.40	0.0057	9969.61	<0.0001	18.82	<0.0001
Red edge	210.83	0.0001	382.93	<0.0001	97.47	<0.0001	4.35	0.0992	259.89	<0.0001	2.29	0.0367
RM	9.83	0.0350	2008.51	<0.0001	89.55	<0.0001	23.31	0.0062	8902.68	<0.0001	15.59	<0.0001

50 DAT for the SVI GM, NDVI, $PSSR_a$, $PSSR_b$, and RM. CRI_{green} was the only nonchlorophyll based SVI of the six but was able to distinguish differences between high *O. nubilalis* treatments and noninoculated controls once, at 50 DAT (Table 8) but was not clearly separable from the low infestation treatment. At 66 DAT, NDVI was the only SVI of the six with detectable differences between high *O. nubilalis* infestation treatments and noninoculated controls. Although MCARI and MOSR both were able to detect differences at 66 DAT among high *O. nubilalis* infestation treatments, low infestation treatments, and noninoculated controls, neither were able to do so earlier.

Discussion

Our results suggest that it may be possible to use SVI derived from airborne hyperspectral imagery for detection of plant stress caused by *O. nubilalis* larvae feeding within corn stalks. SVI detection of plant stress in our manually inoculated second generation *O. nubilalis* plots seemed to peak 5–6 wk postinoculation (Tables 7 and 8) Although none of the SVI examined in this study detected first generation infestation plots in 2004, positive results for 2005 suggest the possibility of early season detection of *O. nubilalis* damage under certain conditions.

Although damage to corn plants during the vegetative growth stage by first generation *O. nubilalis* stalk tunneling can result in stunted plants with smaller and fewer leaves, foliar feeding by *O. nubilalis* larvae that is characterized by small holes and lesions in the leaf tissue, would likely need to be significant to reduce photosynthetic leaf area sufficiently for detection by an airborne sensor. It is plausible that stalk tunneling by multiple larvae or a combination of stalk tunneling and inadequate water availability during the vegetative growth stage will increase the rate at which a plant senesces, making it possible to detect damage by first generation *O. nubilalis* larvae late in the season using airborne remote sensed imagery. However, it will likely be difficult to consistently detect early season *O. nubilalis* damage every year as evidenced by only a single year of positive results. Furthermore, the rate of plant senescence for plants stressed by *O. nubilalis* feeding would likely be intensified through interaction with other stressors such as drought, disease or additional insect damage.

Based on the positive results for the chlorophyll-based SVI (GM, NDVI, $PSSR_a$, $PSSR_b$, and RM) at detecting second generation *O. nubilalis* injury, we propose that the improved spectral and spatial resolution provided by airborne hyperspectral remote sensing is especially effective for detecting changes in

Table 7. Mean spectral vegetation indices for *O. nubilalis* inoculation treatments and noninoculated control by time of image acquisition for second generation *O. nubilalis* inoculated treatments from 2004 Iowa

Date	Days after treatment	*O. nubilalis* generation	Treatment	ARI	CRI_{green}	CRI_{red}	GM	MCARI	MOSR	NDVI	$PSSR_a$	$PSSR_b$	Red edge	RM
9 Aug.	12	Second	Control	1.88a	1.56a	0.96a	1.90a	6.04a	3.73a	0.51a	1.61a	2.13a	2.95a	0.95a
			High	1.89a	1.55a	0.97a	1.92a	5.95a	3.70a	0.52a	1.65a	2.15a	2.96a	0.98a
20 Aug.	23	Second	Control	1.93a	1.50a	0.97a	1.93a	6.82a	3.95a	0.52a	1.65a	2.13a	2.93a	0.90a
			High	1.92a	1.51a	0.96a	1.91a	6.63a	3.90a	0.51a	1.63a	2.10a	2.93a	0.90a
3 Sept.	37	Second	Control	1.51b	1.67a	0.69b	1.55b	4.92b	3.54b	0.41b	1.14b	1.60b	2.89b	0.73a
			High	1.23a	1.72b	0.39a	1.29a	2.83a	2.78a	0.30a	0.75a	1.04a	2.61a	0.59b
17 Sept.	51	Second	Control	1.20b	1.74a	0.39b	1.22b	2.57b	2.86b	0.24b	0.55b	0.79b	2.74b	0.45b
			High	1.07a	1.73a	0.16a	1.09a	1.47a	2.19a	0.17a	0.37a	0.49a	2.43a	0.38a
		SEM		0.01	0.004	0.01	0.01	0.11	0.04	0.01	0.02	0.02	0.02	0.01

Mean SVI with the same letter within each date are not statistically different at 0.05 level of significance.

Table 8. Mean spectral vegetation indices for *O. nubilalis* inoculation treatments and noninoculated control by time of image acquisition for second generation *O. nubilalis* inoculated treatments from 2005 Iowa

Image acquisition date	Days after treatment	*O. nubilalis* generation	Treatment	ARI	CRI_{green}	CRI_{red}	GM	MCARI	MOSR	NDVI	$PSSR_a$	$PSSR_b$	Red edge	RM
1 Aug.	10	Second	Control	1.67a	1.27a	1.40a	1.70a	2.52a	3.78a	0.73a	2.72a	2.51a	3.11a	2.34a
			Low	1.66a	1.27a	1.40a	1.70a	2.50a	3.76a	0.73a	2.72a	2.51a	3.11a	2.35a
			High	1.66a	1.26a	1.39a	1.69a	2.54a	3.79a	0.74a	2.72a	2.51a	3.12a	2.34a
17 Aug.	26	Second	Control	1.61a	1.56a	1.62a	1.60a	2.66a	3.87a	0.74a	2.67a	2.50a	2.95a	2.26b
			Low	1.61a	1.54a	1.61a	1.59a	2.64a	3.86a	0.74a	2.65a	2.48a	2.94a	2.25b
			High	1.64a	1.52a	1.60a	1.55a	2.72a	3.92a	0.73a	2.61a	2.45a	2.94a	2.18a
30 Aug.	39	Second	Control	1.91a	0.52a	1.12a	1.40b	2.69a	3.97a	0.55b	1.99b	2.03b	2.86b	1.63b
			Low	1.91a	0.51a	1.11a	1.38b	2.66a	3.96a	0.54b	1.97b	2.00b	2.84ab	1.61b
			High	1.89a	0.45a	1.04a	1.27a	2.67a	4.00a	0.47a	1.80a	1.84a	2.77a	1.44a
10 Sept.	50	Second	Control	1.94a	1.30b	1.64a	1.27b	4.04a	4.96a	0.70b	2.44b	2.19b	3.06a	1.58b
			Low	1.94a	1.28ab	1.62a	1.26b	4.03a	4.96a	0.70b	2.42b	2.18b	3.05a	1.56b
			High	1.93a	1.21a	1.57a	1.19a	4.01a	4.99a	0.66a	2.29a	2.07a	3.01a	1.43a
26 Sept.	66	Second	Control	1.67a	0.47a	0.75a	0.65a	1.81b	3.22b	0.10b	1.06a	1.13a	2.97a	0.64a
			Low	1.67a	0.46a	0.74a	0.64a	1.75b	3.14b	0.09ab	1.05a	1.12a	2.94a	0.64a
			High	1.66a	0.44a	0.71a	0.60a	1.60a	2.84a	0.08a	1.01a	1.09a	2.89a	0.60a
		SEM		0.01	0.02	0.02	0.01	0.03	0.03	0.005	0.01	0.01	0.02	0.01

Mean SVI with the same letter within each date are not statistically different at 0.05 level of significance.

chlorophyll content caused by plant stress from feeding *O. nubilalis* larvae. In contrast, the SVI (ARI, CRI_{green}, and CRI_{red}) that focused on anthocyanin or carotenoid pigments for detection of *O. nubilalis* feeding stress were not as effective. One likely reason may be that chlorophyll is the primary pigment that determines the amount of light a plant absorbs, and degradation of chlorophyll through catabolism during senescence (Matile and Hortensteiner 1999), or as a result of some other stressor, is an obvious sign of plant health and easily detectable by spectral methods. Plant stress is clearly reflected by changes in chlorophyll concentration, although these changes may be small and may, initially, not be detectable (Blackburn 1998). Until chlorophyll is sufficiently degraded in the leaf, the expression of carotenoid and anthocyanin pigments is masked making detection of either difficult. However, this does not mean anthocyanins and carotenes cannot provide complementary spectral information that may be unique to plants stressed by insect feeding (Blackburn 1998).

Ancillary information, such as spatial heterogeneity of plant pigments, temporal heterogeneity of plant pigment expression and catabolism, canopy structure, and soil type, can provide additional information on individual stressors that may allow insect-related plant stress to be distinguished from nutrient deficiency or disease by remote sensing (GopalaPillai and Tian 1999, Baret et al. 2007, Blackburn 2007). Additionally, spatial heterogeneity of plants exhibiting different plant pigment concentrations over time can help distinguish larger environmental stresses, such as drought, from insect pest or disease stress that initially impact localized areas of a field and may be temporally limited.

This research was conducted on corn plots of same phenology, and were they managed to minimize the physiological response of the plant to other potential stressors, such as nutrient deficiency and weeds. We acknowledge that use of hyperspectral imagery to detect plant stress caused by larval *O. nubilalis* in larger cornfields may be more difficult given the inherent variability of production sized fields especially if there are in-field differences in soil type (Chance 1977, Curran 1985). However, to our knowledge, high spectral and spatial resolution airborne hyperspectral imagery has not been previously used to detect plant stress caused by insect feeding in production corn fields. Unlike imagery acquired by boom mounted or handheld spectroradiometers that are limited by a small FOV and do not typically provide detailed spatial information, airborne hyperspectral imagery is scalable and can provide georeferenced imagery at submeter resolution.

Five of the spectral vegetation indices (GM, NDVI, PSSRa, PSSRb, and RM) used in this study provided consistent, functionally equivalent, results for second generation *O. nubilalis* in 2004 and 2005. In particular, the NDVI and RM distinguished plots inoculated with second generation *O. nubilalis* more frequently than the other SVI. We suggest that for the purpose of identifying plant stress associated with larval *O. nubilalis* feeding in transgenic fields that multiple SVI targeting different aspects of the crop be used to avoid overreliance on a single physiological state. Fundamentally, SVI are designed to minimize variation from nondesired factors and maximize sensitivity to a factor of interest (Daughtry et al. 2000). New SVI for corn that may be developed for this purpose should be sensitive to foliage parameters, such as density, but should not be significantly influenced by other factors, such as soil and atmosphere (Gilabert et al. 1996). However, any crop specific SVI would need to be flexible to account for spectral variation among hybrids.

Current demand for corn in the United States has led to significantly higher corn prices and is expected to contribute to increases in planted acreages of transgenic corn. These added plantings increases the possibility of insect pests developing resistance to Bt corn and emphasizes the need for a detection method ca-

pable of identifying insect related stress in corn. We believe that this study provides evidence that SVI derived from airborne hyperspectral imagery can be used to detect plant stress resulting from larval *O. nubilalis* feeding damage and that this method may provide a means of detecting resistant populations of *O. nubilalis* in production sized Bt corn fields and should be evaluated further.

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